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Refer to the end of the present
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METHOD FOR DIAGNOSING AND/OR MONITORING THE PROGRESSION
OF A CAPILLARY DISORDER AND/OR MEASURING THE EFFICACY
OF A TREATMENT APPLIED TO COMBAT A CAPILLARY DISORDER

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Abstract

The invention relates to a method for diagnosing a capillary disorder and/or monitoring the progression of a capillary disorder and/or measuring the efficacy of a treatment applied to combat a capillary disorder, characterized by the fact that one isolates at least one hair follicle

from a subject, which one incubates in an adequate culture medium for a sufficient time, and by the fact that one assays at least one inflammation mediator, which is connected to the capillary disorder.

According to the method, it is preferred to assay a mediator chosen from the interleukins or the prostaglandins, particularly interleukin 1- α or the prostaglandins E2.

The present invention relates to a method for diagnosing a capillary disorder and/or monitoring the progression of a capillary disorder and/or measuring the efficacy of a treatment applied to combat a capillary disorder.

The expression capillary disorders is defined as any modification of the hair follicle for any reason (for example, modification of gene expression, modification of the metabolism, structural modification of the hair follicle, presence of an exogenous agent (virus, bacteria)), whose consequence is, or potentially will be, a disruption, even extremely minor, of the normal life cycle of the hair follicle and/or its pigmentation.

The applicant has discovered that some capillary disorders present an inflammatory phase which results from the modification of the level of expression of the inflammation mediators.

Thus, after numerous studies, the applicant was able to show that it is possible to use at least one isolated hair follicle to diagnose capillary disorders, and to use this isolated hair follicle for evaluating a subject's risk of suffering said disorders. It is also possible to use this isolated hair follicle to monitor the progression of said capillary disorders and to measure the efficacy of a treatment applied to combat them.

Thus, the invention relates to a method for diagnosing a capillary disorder and/or monitoring the progression of a capillary disorder and/or measuring the efficacy of a treatment applied to combat a capillary disorder, characterized by the fact that one isolates at least one hair follicle from a subject, followed by incubation of this hair follicle in an adequate culture medium for a sufficient time, and by the fact that one assays at least one inflammation mediator, connected with the capillary disorder.

The term hair follicle is defined, according to the invention, as hair or fur. The hair follicle can be intact, that is it can comprise all the parts recognized by a person skilled in the art as components (on this topic, see also: Science des traitements capillaires [Science of Capillary Treatments], Charles Zviak, publisher Masson, 1987). One will cite, for example, that they include among their components: the dermic papilla, the hair bulb, the epithelial sheaths, and the sebaceous gland.

Naturally, the invention is not limited to the intact hair follicle and also concerns any hair follicle which after isolation has not remained intact, preserving only a portion of the constituent parts.

An advantage according to the invention is that it provides a model of diagnosis of capillary disorders which is simple, rapid and effective, and does not necessarily involve invasive steps.

In capillary disorders, those which generally result in the most damaging consequences for the subjects who suffer from them pertain to the state of the scalp hair of the subject.

The method according to the invention thus makes it possible to diagnose capillary disorders pertaining to the state of the subject's scalp hair, and preferably its current or future state.

Thus, the invention allows the determination of the adequate treatment, which may be prophylactic or curative, to limit, or eliminate, said capillary disorder. In addition, the invention makes it possible to monitor the progression of said capillary disorders and to measure the efficacy of a treatment applied to combat them.

In general, these disorders result in a modification of the color of the hair follicles, or in a modification of the density, the quantity or the quality of the hair follicles, resulting, for example, from a growth retardation, a growth stoppage, or a loss of hair follicles, or an excessive growth of the hair follicles.

It is preferred to use the method according to the invention to diagnose at least one capillary disorder and/or to monitor the progression of at least one capillary disorder and/or to measure the efficacy of a treatment used to combat at least one capillary disorder which pertains to the growth retardation or growth stoppage, or to the loss or the coloration of the hair follicles.

If one only examines growth retardation, growth stoppage or loss of the hair follicles, the final consequence for the subject is alopecia to varying degrees, which is known to potentially have aesthetic, psychological and social consequences for the subject concerned.

More particularly, the method according to the invention makes it possible to diagnosis at least one capillary disorder and/or to monitor the progression of at least one capillary disorder and/or to measure the efficacy of a treatment used to combat at least one capillary disorder, which pertains to growth retardation, growth stoppage, or loss of the hair follicles.

The method according to the invention thus makes it possible to diagnose at least one capillary disorder and/or to monitor the progression of at least one capillary disorder and/or to measure the efficacy of a treatment used to combat the alopecia.

The term alopecia covers an entire family of disorders of the hair follicle which result, regardless of the reason, in the partial or general irreversible loss of hair.

One can cite, for example, androgenetic alopecia, alopecia areata (pelade), alopecia totalis, or alopecia universalis.

Without any intent of limiting oneself to any theory of the invention, it appears that some alopecia types pass through at least one inflammatory phase.

Thus it is preferred to use the method according to the invention to diagnose at least one inflammatory phase of the alopecia and to monitor the progression of at least one inflammatory phase of the alopecia and/or to measure the efficacy of a treatment used to combat at least one inflammatory phase of the alopecia and thus to evaluate a given subject's risk of development and/or outcome of alopecia.

The inflammatory phase of the alopecia is characterized by factors including a modification of the level of expression of the inflammation mediators. The risk of alopecia of a given subject can thus be evaluated by the method according to the invention by assaying at least one of the inflammation mediators.

Thus, advantageously, in the method according to the invention, one seeks to assay at least one of the inflammation mediators.

These mediators include the cytokines, particularly interleukin 1- α , interleukin 1- β , interleukin 6, the tumor necrosis factors α and β (TNF- α and β), the chemokines such as interleukin 8 or the monocyte chemotactic activating factor (MCAF), or other chemotactic factors responsible for recruiting lymphocytic, monocytic, Langerhans', or basophilic cells at the inflammatory site, such as the leukotrienes B-4, or other factors involved in the inflammation cascade, such as arachidonic acid, or the prostaglandins, particularly the prostaglandins E2.

Indeed, the applicant has found that in some subjects, particularly those with incipient alopecia, the interleukin level is modified. The interleukin 1- α and interleukin 8 levels, and particularly the interleukin 1- α level, is increased in most of the subjects with incipient alopecia.

It is preferred for the inflammation mediator to be assayed in the method according to the invention to be interleukin 1- α and interleukin 8, particularly the interleukin 1- α level.

The applicant has also found that in some subjects with advanced alopecia, the level of prostaglandins E2 is higher than in others, suggesting an involvement of this mediator in the progression of this disorder. Thus, an early assay result showing any variation in the level of prostaglandins E2 makes it possible to foretell a worsening of the alopecia. It is then also possible to suggest an appropriate treatment.

Thus, the prostaglandins E2 are another inflammation mediator connected with a capillary disorder which can be assayed by the method according to the invention.

The hair follicle used in the method according to the invention can be isolated by dissection, regardless of the dissection technique used, or by epilation, the latter being a preferred variant of the invention.

The dissection can be carried out according to the known standard methods, for example, the method described in "Cultivation of Murine Hair Follicles as Organoides in a Collagen Matrix" (Journal of Investigative Dermatology, 89, No. 4 (1987), 369-379), in which method the follicle is isolated by digestion of the dermis with collagenase, or such as the one described in

European Patent No. EP A 0 434 319, or the one described in the publication by Williams and Stenn (Dakin Williams and Kurt S. Stenn, Dev. Biol. 165, 469-479 (1994)); the above methods involve only mechanical means.

These methods for isolating the hair follicle are used according to the invention when the integrity of the hair follicle must absolutely be preserved. It is preferred to use the technique described in the publication by Williams and Stenn.

Epilation consists of an abrupt separation of the hair follicle and the dermis, generally performed by applying a more or less strong traction to the hair stem of the follicle. After the epilation of the follicle, the follicle can contain all the parts that comprise the hair follicle, but usually a portion of those parts disappear during the epilation.

Epilation is a particularly interesting operational variant to isolate the hair follicle, since it presents the advantages of not being invasive and thus not causing trauma to the subject, and of being simple and rapid to implement.

An additional advantage resides in the fact that the epilation can be performed by the subject himself/herself, and anywhere, so that the subject is not exposed to any additional constraint.

After the isolation, the hair follicle is placed in an adequate culture medium. This nutrient medium consists of at least the elements required for the survival of the hair follicle.

Naturally, it can contain any other element required, for example, for the growth of the hair follicle.

Examples cited include, as culture media which are well-known to a person skilled in the art, the modified Dulbecco MEM medium, the Williams/ medium E, the F12 medium, the HAM [sic; Ham's F12] medium, or RPMI1640, sold by the companies Gibco-BRL, Biomed, Boehringer or Sigma.

One of the advantages of the invention resides in the fact that it makes it possible to obtain an image of the state of the hair follicle at any given time. Thus, after a very short incubation time, one obtains an idea of this state at the time of the isolation. However, generally, the incubation time is determined by the time required for the hair follicle to metabolize the sought inflammation mediator.

This incubation time can range from a few minutes to several days. This incubation time can range from several seconds to several days. As an indication, the incubation time is generally between 5 sec and 96 h, preferably between 12 and 24 h.

The image of the state of the hair follicle at any given time is thus represented by the inflammation mediator which one determines in the method.

For this purpose, the method according to the invention comprises an assay step for the determination of the sought inflammation mediator.

After the incubation, this assay can be performed directly on the culture medium for an inflammation mediator excreted by the cell, or in the hair follicle for an inflammation mediator that is not excreted.

Thus, and more particularly in the case where the inflammation mediator sought is not excreted, one can consider an additional step before the assay, during which the hair follicle is crushed, to make the inflammation mediator to be assayed more accessible.

Naturally, regardless of the implementation variant used for the method according to the invention, any assay method known to a person skilled in the art can be used.

As nonlimiting examples, one can cite the protein assay methods, or nucleic acid assay methods by colorimetry, electrophoresis, reverse transcriptase and amplification by the polymerization chain reaction technique, mass spectrometry, (gas or plate) chromatography, and immunological methods.

In the case where the method is used to assay a deficient expression or overexpression of the inflammation mediator, a step of comparison of the results of the assay with at least one control is carried out after the assay of the sought inflammation mediator.

Usually, a person skilled in the art will easily determine, as a matter of habit, what type of control is needed for the implementation of the method. The control corresponds to the assay of the same inflammation mediator coming from an incubation under the same conditions of a hair follicle isolated in the same manner, where this follicle originates from an individual or a population of individuals.

One of the advantages of the method according to the invention is that it can be used to measure the risks to which a subject is exposed, particularly the risks of developing an alopecia or of advancing an existing alopecia, through one of the inflammation mediators, such as, for example, interleukin 1- α .

Thus, the applicant has developed a method by which, by assaying interleukin 1- α on an isolated hair follicle which is at least kept alive, it is possible to classify the subjects studied as a function of their risk of developing an alopecia, or advancing an existing alopecia.

According to the invention, the subjects can then be classified as a function of their level of the assayed inflammation mediator.

In the case of a capillary disorder which has one component which is an inflammatory phenomenon, one seeks to apply the best suited treatment to combat this disorder. Thus, one will seek to apply a treatment directed against the inflammation mediators. The method according to the invention makes it possible to monitor the efficacy of the applied treatment over time, by regularly assaying during the treatment the inflammation mediator connected with the capillary disorder which one wishes to combat.

Thus, one of the objects of the invention is the implementation of the method according to the invention for the purpose of monitoring the treatment applied to combat a capillary disorder.

Examples will now be given as illustrations; they do not limit the scope of the invention in any manner.

The three examples which will be described show that, among the population of individuals with alopecia, some present, at the level of their hair follicle, a disorder in the production of inflammatory factors (interleukin 1- α and prostaglandin).

The estimation of this inflammatory character by measuring the level of IL1- α in the hair follicle is reproducible over time.

Consequently, the early identification of individuals presenting a hyperproduction of these inflammatory mediators in their follicles after epilation, makes it possible to predict in advance the risk of developing an alopecia and to engage in a therapeutic strategy with the intent of limiting the development of the alopecia.

Example 1:

Study of a group of subjects, pertaining to the evaluation of their risk of developing an alopecia, or of advancing an existing alopecia. Classification of the subjects as a function of their level of interleukin 1- α .

Five hair follicles originating from donors with alopecia were collected in the zone of the vertex. They are immediately incubated in William's [medium] E, sold by the company Gibco BRL (Bethesda, MD, USA) in the presence of antibiotics (penicillin G, 100 units/mL; streptomycin-S, 100 μ g/mL, amphotericin 250 ng/mL) in the amount of 200 μ L of medium per hair follicle from the epilation.

After 20 h, the culture supernatants are collected in a microtube, and then centrifuged for 5 min at 14,000 rpm (Eppendorff centrifuge, model 5415C). The supernatants are then collected in a clean tube, and stored at 4°C.

The concentration of interleukin 1- α is then evaluated from 200 μ L of supernatant using a KIT ELISA Biotrak RPN 2140 kit, sold by the company Amersham (Lee Ulis, France), following the manufacturer's instructions.

| ① Sujets, selon la classification de Norwood* | | |
|---|------|----------------------|
| IL1- α (pg/ml) | | |
| Sujet n° 1 | 21,7 | II |
| Sujet n° 2 | 11,6 | II |
| Sujet n° 3 | 6 | IV |
| Sujet n° 4 | 0 | III |
| Sujet n° 5 | 6 | III |
| Sujet n° 6 | 0 | III |
| Sujet n° 7 | 0 | IV |
| Sujet n° 8 | 35 | II |
| Sujet n° 9 | 0 | III |
| Sujet n° 10 | 0 | III |
| Sujet n° 11 | 19,2 | II |
| Sujet n° 12 | 13,3 | II |
| Sujet n° 13 | 7 | III |
| Sujet n° 14 | 0 | V ou VI ^③ |
| Sujet n° 15 | 10,4 | II |

④ Type d'alopecie selon O'Tar T. Norwood, Male Pattern Baldness : Classification and Incidence, Southern Medical Journal, November 1975, Vol 68, N°. 11.

Key: 1 Subjects, according to the classification of Norwood*

2 Subject No.

3 V or VI

4 * Type of alopecia according to O'Tar T. Norwood, Male Pattern Baldness: Classification and Incidence, Southern Medical Journal, November, 1975, Vol. 68, No. 11.

These results show a good correlation between the alopecia phase according to Norwood and the level of IL 1- α . The subjects whom Norwood considers to suffer from incipient alopecia (class II) indeed present an accentuated inflammatory state, which is confirmed by their high level of IL 1- α (subjects 1, 2, 8, 11, 12 and 15). Some subjects whose alopecia is classified according to Norwood as already advanced (classes III and IV) nevertheless present a moderate level of IL 1- α , which can be explained by the fact that the inflammatory phase of the alopecia is in the terminal phase in these subjects (subjects 3, 5 and 13). The other subjects (4, 6, 7, 9, 10

and 14) present a developed (well-established or very advanced) alopecia, and a low level of IL 1- α , or absence of IL 1- α , indicating that they are in a phase which is posterior with respect to the inflammatory phase.

These results show that the subjects who present a high level of IL 1- α (≥ 7 pg/mL) can be considered to be at a high to very high risk of developing an alopecia or a progression of their alopecia.

The subjects who have a level of IL 1- α which is low or zero (≤ 7 pg/mL) present a low or no risk of developing, in the short term, an alopecia, or they are in an advanced active phase of the alopecia with no inflammatory phase.

Example 2

Study of a group of subjects, pertaining to their risk of being in the inflammatory phase of the alopecia.

Persistence of a high level of interleukin 1- α over time.

Three subjects with alopecia present a high to very high risk of developing an alopecia according to the criteria as defined in Example 1 (production of IL-1 ≥ 7 pg/mL), and six subjects with alopecia who present a low or no risk of developing, in the short term, an alopecia, or who are in an advanced active phase of the alopecia (production of IL-1 ≤ 7 pg/mL), were evaluated three months after the first estimation to verify the persistence of the inflammatory character of their alopecia over time:

| IL 1- α : pg/ml | | |
|------------------------|-----|---------|
| | T=0 | T=3mois |
| 1 Sujet A | 9,9 | 12,7 |
| Sujet B | 8,6 | 16,9 |
| Sujet C | 9,8 | 13,0 |
| Sujet D | 5,2 | 7,3 |
| Sujet E | 4,0 | 8,6 |
| Sujet F | 7,0 | 4,6 |
| Sujet G | 1,0 | 1,5 |
| Sujet H | 2,1 | 2,7 |
| Sujet I | 1,7 | 1,2 |

Key: 1 Subject
2 3 months

It can be observed that for subjects A, B, C, G, H and I, the second analysis reveals that they are in the same risk group as the one identified during the first analysis.

Subjects D and E present either progression or risk of alopecia. At T = 3 months, they are then in the inflammatory phase of the alopecia.

Subject F presents a progression of the risk of alopecia, showing that he probably has gone beyond the inflammatory phase of the alopecia.

Example 3

Study of a group of subjects with alopecia, pertaining to their risk of a progression or worsening of their alopecia; classification of the subjects as a function of their levels of prostaglandin E2. Validation in comparison to a population of subjects without alopecia.

Five hair follicles originating from donors with alopecia are collected in the zone of the vertex. They are immediately incubated in William's [medium] E, sold by the company Gibco BRL (Bethesda, MD, USA) in the presence of antibiotics (penicillin-G, 100 units/mL; streptomycin-S, 100 µg/mL, amphotericin 250 ng/mL).

After 20 h, the follicles collected by epilation from each donor with alopecia (5 hair follicles) are collected in a microtube under an argon atmosphere, and then stored at -80°C.

On the day of the assay, 250 µL of degassed methanol are added to each tube, and then each sample is crushed mechanically (10 rotations) by means of a pestle (tissue grind pestle SZ 20) marketed by the company Kontes (Vineland, New Jersey 08360, USA). The crushed material is then sonicated (20 pulses of 1 sec at 50% amplitude) using a "vibra cell 72434" sonicator, sold by the company Bioblock scientific (Paris, France). The sonicated crushed product is then centrifuged at 4°C for 10 min at 14,000 rpm (Eppendorff centrifuge, model 5415C). The supernatant is then collected in a clean microtube, and lyophilized for 1 h. The lyophilisate is dissolved in 60 µL of phosphate buffer at pH 7.5, supplied in the Kit Biotrak (RPN222), sold by the company Amersham (les Ulis, France). 50 µL of this preparation are then evaluated to determine their PGE2 content using the Kit Biotrak RPN222, following the manufacturer's instructions.

Results

Quantity of PGE2 extracted from different voluntary donors with or without alopecia:

| ① Donneur n° | ② PGE2 (pg/5 épilats) |
|--------------|--|
| | ③ alopéciques (lot 1) ④ non alopéciques (lot 2) |
| a | 4,70 |
| b | 11,40 |
| c | 6,2 |
| d | 1,48 |
| e | 1,99 |
| f | 2,60 |
| g | 3,00 |
| h | 4,40 |
| i | 0,96 |
| j | 2,20 |
| k | 3,90 |

- Key:
- 1 Donor No.
 - 2 PGE2 (pg/5 hair follicles from epilation)
 - 3 Subjects with alopecia (lot 1)
 - 4 Subjects without alopecia (lot 2)

Statistical analyses were performed by comparison of the means using the Student t-test (D. Schwartz/Méthodes statistiques à l'usage des médecins et des biologistes [Statistical methods for physicians and biologists]. Flammarion médecine et sciences 1989).

The null hypothesis (H_0) is formulated as: Lot No. 1 \leq Lot No. 2, in the context of a unilateral test (Lot No. 1 $>$ Lot No. 2), taking into account the fact that the values are not paired.

According to the Student t-test, the H_0 hypothesis is rejected at the threshold of $p = 5\%$. Here $p = 0.0025$.

Conclusion: Since H_0 is rejected, one can conclude that, at a threshold of 5%, the two lots differ significantly; the results obtained for lot 1 are significantly higher than those obtained with lot 2.

Thus, one can conclude that the three subjects with alopecia whose level of PGE2 is significantly elevated have a high probability of worsening their alopecia.

Claims

1. Method for diagnosing a capillary disorder of a subject and/or to monitor the progression of a capillary disorder and/or to measure the efficacy of a treatment applied to combat a capillary disorder, characterized by the fact that at least one hair follicle of said subject is isolated, one incubates this hair follicle in an adequate culture medium for a sufficient time, and one assays at least one inflammation mediator, which is connected to the capillary disorder.
2. Method according to the preceding claim, characterized by the fact that the capillary disorder pertains to the state of the subject's scalp hair.
3. Method according to the preceding claim, characterized by the fact that the capillary disorder pertains to the present or future state of the subject's scalp hair.
4. Method according to the preceding claim, characterized by the fact that the disorder pertains to the growth, the loss or the coloration of the hair follicles.
5. Method according to the preceding claim, characterized by the fact that the disorder pertains to the growth or the loss of the hair follicles.
6. Method according to the preceding claim, for measuring the risks of alopecia and/or of the progression of a subject's alopecia.
7. Method according to the any one of the preceding claims, characterized by the fact that one assays at least one mediator chosen from the cytokines, particularly interleukin 1- α , interleukin 1- β , interleukin 6, the tumor necrosis factors α and β (TNF- α and β), the chemokines such as interleukin 8 or the monocyte chemotactic activating factor (MCAF), or other chemotactic factors responsible for the recruitment of lymphocytic, monocytic, Langerhans' or basophilic cells at the inflammation site, such as the leukotrienes B-4, or other factors involved in the inflammation cascade, such as arachidonic acid, or the prostaglandins, particularly the prostaglandins E2.
8. Method according to the preceding claim, characterized by the fact that one assays at least one mediator chosen from the interleukins.
9. Method according to the preceding claim, characterized by the fact that one assays interleukin 1- α .
10. Method according to Claim 7, characterized by the fact that one assays the prostaglandins.
11. Method according to the preceding claim, characterized by the fact that one assays the prostaglandins E2.
12. Method according to any one of the preceding claims, characterized by the fact that one isolates the hair follicle by dissection.
13. Method according to any one of Claims 1-11, characterized by the fact that one isolates the hair follicle by epilation.

14. Method according to any one of the preceding claims, characterized by the fact that the culture medium comprises at least one element necessary for the survival of the hair follicle.

15. Method according to the preceding claim, characterized by the fact that the culture medium comprises at least one element necessary for the growth of the hair follicle.

16. Method according to any one of the preceding claims, characterized by the fact that incubation time is between 5 sec and 96 h.

17. Method according to the preceding claim, characterized by the fact that the incubation time is between 12 and 24 h.

18. Method according to any one of the preceding claims, characterized by the fact that one assays the sought inflammation mediator directly in the culture medium.

19. Method according to any one of Claims 1-7, characterized by the fact that one assays the sought inflammation mediator in the hair follicle.

20. Method according to the preceding claim, characterized by the fact that, before the assay of the sought inflammation mediator, one proceeds to the crushing of the hair follicle.

21. Method according to any one of the preceding claims, characterized by the fact that a comparison stage is performed, comparing the results of the assay to at least one control, after the stage of assaying the sought inflammation mediator.

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SEARCH REPORT
established on the basis of the most
recent claims filed before the start
of the search

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| Category | Citation of document with indication where appropriate, of relevant passages | |
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| CATEGORY OF CITED DOCUMENTS | | |
| X | Particularly relevant if taken alone | T Theory or principle underlying the invention. |
| Y | Particularly relevant if combined with another document of the same category | E Earlier patent document, but published on, or after the filing date. |
| A | Technological background. | D Document cited in the application. |
| O | Non-written disclosure. | L Document cited for other reasons. |
| P | Intermediate document. | & Member of the same patent family, corresponding document. |